Editorial

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

The invaders and the barrier

Jean-Michel Pawlotsky*

National Reference Center for Viral Hepatitis B, C and Delta, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France; INSERM U955, Créteil, France

See Article, pages 41–48

Barriers exist to protect. The first images of World War II show the invaders breaking a barrier at the border of Poland, signifying the end of a peaceful world and the beginning of chaos... This is analogous to viral resistance to antiviral drugs. The invader is the drug-resistant virus; this virus preexists as poorly fit, minority viral populations. The "barrier to resistance" of a drug or a drug combination prevents their outgrowth in the presence of the drug(s), thus preventing virological breakthrough, disease progression, and eventually severe complications. If the barrier to resistance is high enough, resistant viral variants are not selected and do not grow; if it is not, they rapidly fill in the replication space and become the dominant (or exclusive) viral population associated with high-level replication.

The main components of the barrier to resistance *in vivo* are: (i) the "genetic barrier to resistance", defined as the number of amino acid substitutions needed for a viral variant to acquire full resistance to the drug in question. If a single substitution is sufficient to confer high-level resistance, then the drug is considered to have a low genetic barrier to resistance, while the need for three or more substitutions represents a high genetic barrier; (ii) the "*in vivo* fitness" of the resistant viral variant population, defined as its ability to survive and grow in the replicative environment; (iii) drug exposure, defined as the drug concentration achieved *in vivo* relative to the 50% and 90% inhibitory concentrations (IC) and efficient concentrations (EC) [1].

A number of direct acting antiviral (DAA) drugs are in development for the treatment of chronic hepatitis C virus (HCV) infection. Two NS3/4A protease inhibitors, telaprevir and boceprevir, have been recently approved in combination with pegylated interferon (IFN)-α and ribavirin for the treatment of genotype 1 chronic hepatitis C [2–5]. Other DAAs are at various stages of preclinical to late clinical development. They can be schematically classified into two groups, according to their barrier to resistance. Drugs with a low barrier to resistance include first-generation NS3/4A protease inhibitors (e.g. telaprevir and boceprevir and numerous other molecules in development), NS5A inhibitors, and non-nucleoside inhibitors (NNI) of HCV RNA-dependent RNA polymerase (RdRp) [6]. Their administration as monotherapies has been reported to be associated with early virological breakthroughs due to the selection and subsequent outgrowth of fit resistant viral populations carrying one or several substitutions that confer resistance to the drug [7–9]. Drugs with a high barrier to resistance include nucleoside/nucleotide analogue inhibitors of HCV RdRp, cyclophylin inhibitors (drugs that target a host cell protein involved in viral replication), and potential second-generation NS3/4A protease inhibitors. They can be administered alone for weeks without any virological breakthrough due to resistance in the majority of cases [10,11].

Prevention of resistance, particularly when drugs with a low barrier to resistance are used, is based on the combination of several molecules that are potent and have no cross-resistance. No cross-resistance means that each member of the combination is fully active on viruses that are resistant to the others. This concept was the basis for combining NS3/4A protease inhibitors (or other DAAs with a low barrier to resistance) with pegylated IFN-α and ribavirin. Unfortunately, in patients who do not respond adequately to IFN-α and ribavirin, treatment fails and as a result DAA-resistant variant populations grow [1]. In order to cure infection without selecting for resistance, it is also possible to combine potent DAAs without cross-resistance, with the double goal of achieving better antiviral efficacy and substantially increasing the barrier to resistance. Attempts with combinations of drugs with low barriers to resistance, such as an NS3/4A protease inhibitor with an NS5A inhibitor, or an NS3/4A protease inhibitor with an NNI, have been disappointing [12,13]. Although cure was achieved in a few patients receiving the former combination [14], the rates of failure due to selection of viral variants bearing substitutions at both drug target sites were frequent in these studies, suggesting that the barrier to resistance of a combination of two HCV drugs with low barriers to resistance is not dramatically greater than that of each drug alone.

There are four distinct groups of NNI inhibitors of HCV RdRp in development. Each targets a different allosteric site at the surface of the enzyme, and they have been reported to have different resistance profiles *in vitro*, without cross-resistance. Thus, although targeting the same viral enzyme, NNIs from different classes could theoretically be combined together. In this issue of the *Journal of Hepatology*, Delang *et al.* report their assessment of the antiviral potencies and resistance selection profiles of members of three of the four NNI groups [15]. These compounds were tested alone and in double or triple combination in replicon-harboring Huh7 cell lines, the usual model for this type of

☆ DOI of original article: 10.1016/j.jhep.2011.04.016.

* Tel.: +33 1 4981 2827; fax: +33 1 4981 4831.
E-mail address: jean-michel.pawlotsky@hmn.aphp.fr

experiments. The authors confirmed the low barrier to resistance of each NNI alone and the lack of cross-resistance between them. They also showed that these drugs have additive antiviral effects *in vitro*.

When a stepwise, long-term procedure was used, the authors were able to select variants resistant to each pair-wise combination, which carried amino acid substitutions conferring high-level resistance to both tested NNIs on the same strain. Triple resistant replicons were also generated, starting from a replicon that was already resistant to two NNIs and was subsequently exposed to the third one. The triple-resistant replicons harbored substitutions conferring resistance to the three drugs tested. It was also cross-resistant with the fourth class of NNIs, not used in the experiments [15]. Whether such triple resistant variants naturally preexist in infected patients remains unknown. Short-term replication models, such as replicons in Huh7 cell lines, could underestimate the extent of HCV variability encountered in patients who have been infected for decades. Indeed, it has been recently suggested, based on mathematical modeling, that in HCV-infected patients, all possible single and double mutants are generated multiple times each day, all viable single and double mutants that confer drug resistance preexist and may compete with the wild-type virus during therapy, and triple mutants can be selected by sequential mutations when single or double mutants replicate [16]. It is therefore highly likely that HCV variants that are resistant to three drugs preexist at baseline in a substantial proportion of patients, or that they can be generated through replication of double-resistant viruses.

Resistance to DAAs is often feared as the main cause of treatment failure with new HCV therapies. This is not the case with the triple combination of a DAA with pegylated IFN-α and ribavirin, during which treatment failure results of an inadequate response to IFN-α that favors the outgrowth of resistant viral variants selected by the DAA [1–5]. In contrast, control of resistance will be key during the era of all-oral, IFN-free regimens. Indeed, antiviral potency and a high barrier to resistance are required to ensure that inhibition of HCV production is sustained for a sufficient amount of time in order for every infected cell to clear the remaining viruses. If any viral population is not controlled by the drug combination, it replicates, is produced, infects new cells and leads to treatment failure. Intuitively, the best way to prevent such failure is to include at least one drug with a high barrier to resistance, such as a nucleoside/nucleotide analogue or a cyclophylin inhibitor, in any combination of HCV DAAs. However, these drugs have not yet reached the market and the results of long-term combination studies including one or two nucleoside/nucleotide analogue(s) are awaited.

What will be the role of NNIs in this context? Recent results with the two most advanced in development NNIs, tegobuvir and filibuvir, have been disappointing: no difference was observed between the triple combination of different doses of the NNI with pegylated IFN-α and ribavirin *vs.* pegylated IFN-α and ribavirin alone [17,18]. Nevertheless, this failure appears to be principally related to the lack of antiviral potency of these compounds rather than to their barrier to resistance, which is not fundamentally different from that of telaprevir or boceprevir. It is therefore possible that more potent NNIs could prove to be useful in combination with pegylated IFN-α and ribavirin.

What the study by Delang *et al.* teaches us is that combinations of NNIs are unlikely to be helpful, even if the molecules belong to different classes, have different target sites and no cross-resistance. *In vitro* results in the replicon system have proven to be accurate in predicting resistance *in vivo*; thus, single, double, and triple-resistant variants are likely to be selected early by NNI combinations in infected patients. How will we use NNIs in IFN-free regimens in the future? Ideally, in combinations including at least one drug with a high barrier to resistance (e.g. a nucleoside/nucleotide analogue, a cyclophylin inhibitor, or a second-generation NS3/4A protease inhibitor with an improved resistance profile compared to first-generation ones). Nevertheless, if these drugs are potent enough, they should, at least theoretically, be able to block virus production without resistance emergence for long enough for the virus to be cleared by host cells without the need for other drugs. If this is the case, drugs with a narrow genotype coverage and a low barrier to resistance, such as NNIs, may not be absolutely required in the HCV drug armamentarium. This question will be solved by ongoing and future clinical trials.

Overall, trial designers and clinicians should remember that, if resistance is not a major threat in patients treated with the triple combination of a DAA with pegylated IFN-α and ribavirin, since the final outcome depends mainly on the IFN response, its prevention will be key in the design of all-oral treatment strategies based on DAA combinations. Only combinations with a high enough barrier to resistance should be envisaged, as they are the only ones that can ensure sustained inhibition of viral production for the time needed for host cells to get rid of the virus. As in any war, the final victory depends on the allies you chose. Our mission is now to find the best alliance to keep the invaders out. This is the price to pay for a peaceful world, i.e. a world without hepatitis C.

Conflict of interest

The author has received research grants from Gilead and Roche. He has served as an advisor for Abbott, Anadys, Biotica, Boehringer-Ingelheim, Bristol-Myers Squibb, DebioPharm, Gilead, GlaxoSmithKline, Idenix, Janssen-Cilag, Madaus-Rottapharm, Schering-Plough/Merck, Novartis, Pfizer, Pharmasset, Roche, Vertex and Virco.

References

[1] Pawlotsky JM. Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. Hepatology 2011;53:1742–1751.
[2] Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1207–1217.
[3] Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364:2405–2416.
[4] Poordad F, McCone Jr J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195–1206.
[5] Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med 2011;364:2417–2428.
[6] Pawlotsky JM, Chevaliez S, McHutchison JG. The hepatitis C virus life cycle as a target for new antiviral therapies. Gastroenterology 2007;132:1979–1998.
[7] Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007;132:1767–1777.
[8] Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010;465:96–100.
[9] Mori J, Hammond JL, Srinivasan S, Jagannatha S, van der Ryst E. Genotypic characterisation of filibuvir (PF-00868554) resistance in patients receiving

four weeks co-administration of filibuvir with pegIFN/RBV (12-week analysis). J Hepatol 2010;52:S15.
[10] Le Pogam S, Seshaadri A, Ewing A, Kang H, Kosaka A, Yan JM, et al. RG7128 alone or in combination with pegylated interferon-alpha2a and ribavirin prevents hepatitis C virus (HCV) replication and selection of resistant variants in HCV-infected patients. J Infect Dis 2010;202:1510–1519.
[11] Coelmont L, Gallay P, Bobardt M, Kaptein S, Paeshuyse J, Vliegen I, et al. Particular in vitro anti-HCV activities and resistance profile of the cyclophylin inhibitor DEBIO-025. J Hepatol 2009;50:S36.
[12] Zeuzem S, Buggisch P, Agarwal K, Manns MP, Marcellin P, Foster GR, et al. Dual, triple, and quadruple combination treatment with a protease inhibitor (GS-9256) and a polymerase inhibitor (GS-9190) alone and in combination with ribavirin (RBV) or PegIFN/RBV for up to 28 days in treatment-naïve, genotype 1 HCV subjects. Hepatology 2010;52:400A.
[13] Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib RH, et al. Combination therapy with BMS-790052 and BMS-650032 alone or with PegIFN/RBV results in undetectable HCV RNA through 12 weeks of therapy in HCV genotype 1 null responders. Hepatology 2010;52:877A.
[14] Lok A, Gardiner D, Lawitz E, Martorell C, Everson G, Ghalib R, et al. Quadruple therapy with BMS-790052, BMS-650032 and PEG-IFN/RBV for 24 weeks results in 100% SVR12 in HCV genotype 1 null responders. J Hepatol 2011;54:S536.
[15] Delang L, Vliegen I, Leyssen P, Neyts J. In vitro selection and characterization of HCV replicons resistant to multiple non-nucleoside polymerase inhibitors. J Hepatol 2012;56:41–48.
[16] Rong L, Dahari H, Ribeiro RM, Perelson AS. Rapid emergence of protease inhibitor resistance in hepatitis C virus. Sci Transl Med 2010;2:30ra32.
[17] Jacobson I, Pockros PJ, Lalezari J, Lawitz E, Rodriguez-Torres M, DeJesus E, et al. Virologic response rates following 4 weeks of filibuvir in combination with pegylated interferon alfa-2a and ribavirin in chronically-infected HCV genotype 1 patients. J Hepatol 2010;52:S465.
[18] Lawitz E, Jacobson I, Godofsky E, Foster GR, Flisiak R, Bennett M, et al. A phase 2B trial comparing 24 to 48 weeks treatment with tegobuvir (GS-9190)/PEG/RBV to 48 weeks treatment with PEG/RBV for chronic genotype 1 HCV infection. J Hepatol 2011;54:S181.